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CULTIVATION OF A PATHOGENIC FUNGUS WHICH EXHIBITS BOTRYOID AND LEUCOCYTELIKE PARASITIC FORMS [1]

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SIX PLATES

In the Philippine Islands, as in other tropical regions, the material available for the study of fungus infections is more abundant than in most other parts of the world. Not only are mycotic lesions very common, but it appears that there are here to be found new types of infectious fungi, some of which, at least, present features that have rarely or never been described as occurring among such organisms.

The purpose of the present communication is to report a recently isolated fungus which possesses a remarkable range of morphologic variation and which is, perhaps, related to, or possibly itself is, the etiological factor of certain conditions whose cause is not understood. In one of its phases this organism produces minute endospores, somewhat like those of bacteria, but quite different from any of the recognized spores of fungi. Another feature is the resemblance of forms which the fungus may assume to certain body cells, particularly the lymphoid and plasma cells. These leucocytelike parasites occur in the active lesions, and in certain cultures their development can be followed. These and other features that the fungus presents in its various phases and modes of development appear not to be peculiar to this fungus alone and seem of considerable interest not only from the standpoint of the pathologist, but also from that of the mycologist.

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The case from which this fungus was isolated was encountered among a number of leper suspects, among whom unfamiliar types of skin infections are occasionally to be seen. This individual, who presented multiform lesions of several types, proved to be nonleprous; he was, however, kept under observation for some time. The principal features of the case, so far as they can be determined, are as follows:

CASE HISTORY

The patient is a male Filipino (Tagalog), laborer, aged about 30 years, resident in Manila for some years. He says that in July or August, 1915, an ulcerative lesion appeared below the left elbow. This healed, but soon reappeared, involving a wider area, beyond which it spread slowly. At about the same time a number of similar spreading lesions appeared simultaneously on other parts. The patient experienced a chill at the time of onset, but thinks that he had no fever. These lesions spread over considerable areas as evidenced by the cicatrices, some of which are of considerable thickness and resemble keloids (fig. 5). About four months later numerous small, scattered lesions began to appear, said to have directly followed a three-day course of some medication.

Upon examination (June 6, 1916) the lesions are seen to be very numerous and varied. On the legs and feet there seems to be little normal skin left. The hands and forearms are also very much involved.

Small, early lesions are present in great numbers over the back (fig. 1), forearms (fig. 4), and legs and to a less extent on the arms, thighs, and shoulders. These are very superficial, erythematous, slightly raised, and somewhat indurated areas, irregular in size and outline, in which are numerous fine papules usually pinhead-sized or smaller. These are not tender on pressure. In and immediately about the areas, except over the papules, the epidermis is pigmented, sometimes to a deep brown. It was probably the peculiar appearance of these small lesions, together with a nodular induration of the lobes of the ears (fig. 2), that had caused the patient to be submitted for examination as a suspected leper.

The small lesions seem more commonly to subside, leaving slight scars or disappearing completely. Over the wrists and hands, feet and ankles, the elbows and the knees, and even on the upper margins of the ears (fig. 2), numbers of them have progressed to form rather prominent verrucæ, which vary in color from white to a dark gray. These apparently develop by a low-grade inflammatory hyperplasia.

A more striking type of lesion, comparatively acute, which apparently originates with the common small lesion, is a relatively large frambœsial nodule (fig. 3), which extends over a part or the whole of a papular area, often encroaching on the adjacent skin. These lesions are abruptly raised and usually oval, measuring from 1 to 2 centimeters in width and from 1.5 to 2.5 or 3 centimeters in length. The covering epidermis is thin, shiny, and transparent, and thin scales usually form upon it. It is apparently easily injured. Beneath it, in the more or less dark gray to purplish gray newly formed tissue, are seen red pinhead-sized points suggesting vascular spaces and others which are pale gray to yellowish, apparently due to necrosis or suppuration. A somewhat similar acute lesion sometimes develops by recurrence in scar tissue (fig. 5, indicated).

These nodules upon incision usually appear much less than normally sensitive. The tissue is very friable and tears readily. Incised wounds heal promptly and cleanly unless secondarily infected. Such an infection is apparently not uncommon and results in more or less persistent ulcers, which are shallow, do not increase in size or do so slowly, exude constantly, and heal reluctantly. Excess scar formation has followed as a rule.

During a period of two months' observation the changes that took place were of minor importance. As a whole, more and more of the skin surface was being encroached upon. The patient's general condition was fairly good, though he was slightly anæmic and below normal in strength.

STUDY OF ORGANISM

At various times smears and cultures were made, and on three occasions tissue was excised for study. After several unsuccessful attempts to cultivate it, the fungus was recovered in its free-growing form. In the earlier cultures there were isolated from certain acute, unbroken lesions a nonhæmolytic *Streptococcus pyogenes* and a delicate diphtheroid. In view of the findings of Rosenau(9) in erythema nodosum, these were studied somewhat fully, but they proved not to be significant except as secondary invaders.

In the earlier cultures the fungus was not recognized, though certain of its forms were later found to have been present in some of the preparations from these cultures.

In describing the various appearances of the organism, it seems advantageous to apply tentatively selected names to forms which, it appears, are not covered by any accepted terminology. These are *a*, the "basic parasitic form," a simple, usually round, densely staining, more or less nucleuslike body, from which other forms develop; *b*, the "entire-cell form," which is evolved by the development of a distinct protoplasm about the basic form; *c*, the "spore-granules," produced under conditions of semiparasitism, which are given this qualified designation because they are very different from the usual spores of fungi and, to a less extent, from common bacterial spores; and *d*, the "gelatinous body," another semiparasitic form, which is a peculiar cultural development of the basic form.

HISTOPATHOLOGY

The tissue forms of the parasites were first found in smears and sections of the soft, granular tissue of a fairly recent, uncomplicated lesion. A small piece was removed and placed in Zenker's fluid; cultures were made from the cut surface. In these cultures there later appeared the first recognized multiplication of the fungus.

The tissue studied was from the nodule proper and is composed of epidermis and newly formed inflammatory tissue. None of the cutaneous gland structures are present. The epidermis is intact, though it is somewhat thin and irregular and, at a few points, as shown in figs. 8 and 9, is invaded by scattered parasites. Throughout the sections vascular channels are very numerous. Here and there, usually close under the epidermis, are areas so vascular and blood-filled as to suggest hemorrhage (fig. 6). In the large, irregular, more or less cavernous spaces, which are usually lined with endothelium and filled with normal erythrocytes, are numbers of leucocytes and apparent forms of the fungus. The lymphatic channels are numerous and prominent in the deeper layers of the tissue. Everywhere there is evident an extensive leucocytic infiltration, particularly in and about the vascular areas (fig. 7). Here are polymorphonuclear leucocytes and, more numerous, cells of the "round-cell" types. In occasional small, active areas are numerous large endothelial leucocytes, occurring usually within blood channels, as in figs. 7 and 10. Most of them, actively phagocytic, contain numbers of parasite bodies. Many of the parasitic cells, even after prolonged study of the cytology of the lesions, are impossible of differentiation from body cells.

In the less active foci are not a few eosinophiles, which have always been observed in the smears from this type of lesion. There is no evidence of pigment deposit in the sections.

By comparing the bodies seen here with certain of those seen in preparations from cultures, the apparent forms of the parasite in the lesion may be determined.

FORMS OF ORGANISM IN TISSUE LESION

The basic forms are simple, small, round, sometimes oval and occasionally crescentic or signet-ring-shaped bodies which, unless degenerated (as in fig. 10), stain intensely by Gram's and general nuclear stains. Occasionally one may find within a phagocyte several organisms which are bean-shaped. Some are found to be slightly acid-fast. The smaller vary in size from mere granules, hardly larger than the ordinary staphylococcus (figs. 8, 9, and 14), to bodies 3 or 4 microns in diameter (figs. 8 to 14). These show no apparent structural differentiation. Practically all of the phagocytized parasites and the smaller of those that are free are of this type. The entire-cell form is produced by the development about the free-lying basic forms of protoplasmic envelopes (see especially figs. 8, 9, 13, and 14). In sections these bodies often measure from 5 to 6 microns in diameter.

Multiplication.—Having attained their maximum size, the entire-cell forms sometimes undergo a simple segmentation, during which the nuclear mass becomes irregular in outline (fig. 10) and is broken up to form botryoid groups of from 5 to 10 small, round, basic forms of different sizes, the individuals of which are ultimately liberated by dissolution of the protoplasm (figs. 8 and 14, *c*). There is no regularity in the number of small forms produced. The segmented parasite in fig. 14 is of unusually large diameter. These groups are not numerous and have been found only in the superficial, more active areas. More common than this segmentation is multiplication by the simple fission of a nuclear body into parts, equal or unequal. This may occur even in the smaller parasites (14, *b*) and occasionally is seen even among the phagocytized bodies.

In the active foci the free-lying forms are particularly numerous, occurring in blood and lymph spaces and among the tissue cells. Infrequently they are seen, as in figs. 13 and 14, invading areas of the epidermis without cellular reaction.

As the parasites do not stain differentially, their recognition both in tissue sections and in smear preparations is dependent in part upon morphology, the outline of the nuclear body usually being strikingly clear-cut, and particularly upon their intensity of staining. In view of their frequently close resemblance to lymphoid and plasma cells and of the intensity with which injured tissue-cell nuclei stain, this is at best an uncertain criterion. The more solid bodies retain Gram's stain more intensely than do tissue nuclei, but a clear differentiation is not possible. There has nowhere been found evidence of capsule formation, the material which develops about the free-lying forms being distinctly protoplasmic in its staining reaction.

CULTURAL FEATURES

Slight multiplication of this organism in cultures on solid media seems always to occur, provided tissue elements are present, but on all except a few media it soon ceases. In smears from such cultures, which appear sterile, the forms that result from this brief multiplication may be easily passed over without recognition, particularly after staining by Gram's method (figs. 15, 16, and 17). Many of the appearances which it may assume are so dissimilar that some of the forms might well pass unrecognized in the search for others. The principal modes of development as they have been observed will be described separately.

DEVELOPMENT OF PARASITIC FORMS ON BLOOD-SMEARED AGAR

If material obtained by scraping an incised, active lesion be planted in a drop or two of blood, whole or defibrinated, smeared on an agar slant, there ensues a course of development some features of which are not to be seen under other conditions.

The homogeneous basic forms, which are slightly blue after Giemsa's stain, enlarge somewhat and stain more deeply. Sometimes the entire-cell form becomes prominent (fig. 18).

Spore-granules.—Within the "nuclear" body of the enlarged parasite, whether it be endowed with protoplasm or not, there develop a few extremely small, lighter points, due to the earliest appearance of the small hyaline spore-granules (fig. 19, upper parasite). The color of the cell now becomes distinctly purplish in Giemsa-stained preparations, more closely resembling a degenerating leucocyte nucleus. The spore-granules enlarge and become more numerous (fig. 19, lower parasite), appearing as minute, round, oval, and rod-shaped bodies.

The outline of the containing cell soon becomes indefinite, and the whole appears soft (fig. 20). Smears occasionally show groups of the spore-granules that are compactly massed (fig. 21). When liberated the spore-granules usually become indistinguishable in smear preparations, though they may be present in large numbers (fig. 22). After Gram's stain they retain the safranine counterstain and may be tentatively identified by their higher refraction. Giemsa's stain does not color them as a rule. After Loeffler's blue the larger are often easily discernible by their clear, light, refractile blue.

The formation of these spore-granules seems an extremely simple process. There is no definite segmentation, nor is there any apparent mechanism governing the number or size. They appear much as might crystals separating out of a protoplasmic mass and occur only within the nucleus until this is no longer demarked. They have not been observed in the tissue sections. While they seem to be produced for multiplication only, comparatively very few are capable of development under the conditions obtaining. In the free blood of these cultures a few (as in the center of fig. 22) enlarged into typical basic forms. In occasional cultures the viable granules, before developing into the leucocytelike bodies and cells, become rod-shaped or slightly curved, often appearing as small bacilli (fig. 23). Prominent in most of these half-grown and larger organisms is the appearance of one or two small metachromatic granules, often placed as bars across the organism. This was the first form to be recognized as developing in the cultures and at first was misunderstood. The more mature cells are usually oval (figs. 24 and 25), and after Giemsa's stain the protoplasm presents a clear, delicate, robin's-egg blue and the metachromatic granules and masses, usually of a clear red, are prominently in contrast. This type of cell develops into the leucocytelike form, with or without protoplasm.

DEVELOPMENT OF PARASITIC FORMS ON MEDIA WITHOUT FREE BLOOD

In tissue scrapings planted on ordinary media without smeared blood there occur forms of early multiplication, most of which might well be mistaken for products of degeneration of inoculated tissue. In smears stained by Gram's there is often to be seen only amorphous material containing a few Gram-positive granules and small, round, or oval bodies (fig. 15). There often are more or less numerous small masses (8 to 12 microns in length), which may be either body or parasite cells (fig. 16) that, because of their rather hyaline, amorphous appearance, may be mistaken for fragments of débris. In some of these cultures the large gelatinous forms appear, but these are not constantly found.

Multiplication of the organisms in these cultures persists only so long as there is tissue substance present; consequently the organisms soon die out.

DEVELOPMENT IN PLANTED TISSUE BITS

Here the parasitic forms sometimes enlarge and quickly produce hyaline spore-granules. Often, however, they are transformed into the very much larger, gelatinous-appearing masses noted, which have developed fully only within pieces of planted tissue. These may be unstained and scarcely recognizable after Gram's stain (fig. 17).

Gelatinous bodies.—In an occasional preparation one may trace the transition of the basic form to small gelatinous bodies (fig. 26) and the enlargement of these. In some cultures those of medium size seem firm and stain deeply, as though the contents were thick and gelatinous, though in more instances they appear to be softer.

Under some conditions the gelatinous bodies develop vast numbers of the small, hyaline spore-granules. At other times the spore-granules and deeply staining basic forms, often very small, develop simultaneously. The latter at times appear in large numbers, with or without the hyaline granules. In fig. 28 is a group of basic-form bodies which developed from such a gelatinous mass as shown in fig. 27. In fig. 29 is an unusual group of closely packed "gelatinous" forms, now thin and vesicular, all of which contain numbers of the basic granules and bodies.

The most favorable condition for this development is cultivation on banana agar at tropical room temperature, about 30°.[2] On such media the basic masses develop extensively and irregularly, assuming bizarre forms (fig. 30). These represent the earliest attempts of the parasitic forms to produce a mycelium. They are to be seen after several days of cultivation. The deeply staining figures become larger and more irregular from day to day and finally grow out of the containing cell and become definitely mycelial. After fifteen days or more, what remains of the tissue bit within which this development occurs appears whitish and delicately fuzzy with hyphous growth. Smears now show mycelium and great numbers of calcium oxalate crystals.

One feature of smears from banana cylinders is the occurrence of vesicular bodies (fig. 31), which might be thought to develop from the gelatinous form of the parasite. They usually are, in reality, cells of the banana pulp and are sometimes of interesting appearance. One podlike cell shown in fig. 32 had ruptured; one with an unusually delicate wall is shown in fig. 33 doubled upon itself. It is possible that the body in fig. 30 is such a banana cell within which basic parasitic forms of the fungus are developing, though this cannot be asserted.

CULTURAL FEATURES OF MYCELIAL FORM

Once the mycelial type of growth has been established, subcultures grow rapidly and very luxuriantly on practically all media, often growing up along the glass of the test tube within a few days.

On solid media.—The downy, pure white growth is a tangled mycelium with many long, straight hyphæ radiating from the inoculation point (figs. 37 and 38). After a day or two it becomes tangled, appearing as if small fluffy snowflakes had become entangled in the loosely interwoven mass (fig. 36).

On glycerin, dextrose, and maltose agars the growth is particularly rapid and luxuriant. On potato and banana plugs it grows less luxuriantly, but on banana agar it develops very rapidly (compare figs. 37 and 38). Any of these cultures, when a few days old, have a typical "yeasty" odor which is striking. This I have never noted in cultures of any other mycelial fungus.

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[2] Banana media, whether cut cylinders or banana pulp (10 per cent or more), in ordinary agar or bouillon, must be sterilized intermittently (Arnold sterilizer) and not autoclaved. The use of this material was suggested to me by Dr. J. A. Johnston, of this institution, who knew of its previous use here by Dr. F. Schmitter, of the United States Army Medical Corps, in the study of pathogenic fungi. In what form he used it has not been learned, as no report of such work has appeared under his name.

After several days the aërial growth for the most part collapses and the surface appears dry and dusty over a compact, firm surface layer. The agar itself becomes gradually clouded by down growth of the mycelium and assumes a yellow to light brown tint.

In fluid media.—In the depths of ordinary bouillon the fungus grows in loose, flocculent masses. In glycerin bouillon, whether 0.5 or 10.0 per cent, the growth is very slow, occurs in much smaller, more compact white masses than in other bouillons, and is always deep in the medium.

In most fluid cultures the greater part of the growth is on the surface where, in a few days, a thick pellicle is formed. The upper surface is at first deeply covered with fine aërial hyphæ which, as on agar, soon collapse (fig. 40). In a few weeks the bouillon assumes a deep amber color.

Growth is by far most abundant on banana bouillon with or without glycerin (fig. 39). The layer is firm, the under surface is smooth, and when torn there appears below the superficial mycelium a distinct membrane, about 1 millimeter thick, which is sharply demarked, firm, homogeneous, and yellow.

Growth is markedly greater at room than at incubator temperature. Anaërobiasis is also very unfavorable to active growth, often inhibiting it completely.

MORPHOLOGY OF MYCELIAL FORM

On solid media.—In the rapidly growing cultures the young hyphæ are rather thin and long and are branched at rather infrequent intervals. Septation is seen only in old filaments (fig. 43). In the aërial hyphæ vacuoles develop (fig. 41), and the protoplasm is reduced to narrow transverse bands somewhat suggestive of septa which determine the points at which the filament separates into individual cells. This process of maturation and dissociation may be complete in two or three days.

In the separated elements, as in figs. 34 and 35, there sometimes is evident a tendency to polar accumulation of the protoplasm. Hyaline granules often appear, and large and small metachromatic granules are common. After a time some of these entire segments become hyaline (fig. 42).

Upon transplanting these elements to fresh media, development usually begins within three or four hours by a lateral pseudopodic offshoot. Sometimes two and, rarely, three of these develop from a single mother cell, which becomes vacuolated and remains (as in figs. 41 and 42) in direct connection with the growing hyphæ.

In fluid media.—In plain bouillon the mycelium which is in the depths remains filamentous, though the aërial growth undergoes separation. After a few days the protoplasm of most of the submerged filaments condenses to form small, highly refractile granules (figs. 43 to 45). At the same time there may be formed short, irregular hyaline filaments which are faintly yellow, to massing of which the brittleness and deep yellow color of the tough yellow membrane which develops on banana bouillon are due. Rarely in old bouillon cultures there appear large chlamydospores (figs. 44 and 45), which develop terminally on apparently undifferentiated hyphæ.

A constant feature, even in young cultures, is the production of crystals which are usually flat, six-sided, and faintly yellowish, though under some conditions they have the typical "envelope" forms of calcium oxalate. These crystals are sometimes present in great numbers.

PATHOGENICITY FOR ANIMALS

As yet no extensive study has been made of the lesions which develop after inoculation of cultures of this mycelium into laboratory animals. Suffice it for the present to say that lesions not simply referable to the presence of the foreign material and which contain typical tissue forms of the parasite develop upon subcutaneous inoculation of fairly large doses. One rabbit which was inoculated intravenously died after twenty hours. There was found a peritonitis and an early plastic pericarditis, and in the exudates were great numbers of a modified entire-cell form of the organism. In some of the cultures from this animal on ordinary media the mycelial growth did not develop, while in others it appeared after three days.

SUMMARY AND DISCUSSION

From a case of mycotic dermatitis presenting multiform, widely distributed lesions, there has been isolated on special media, after several unsuccessful attempts, a fungus which is thought not to have been previously described. The present description is based on the observations made during its isolation from the only case of this nature yet encountered and during the few months since its recovery. Intensive study of numerous cultures has clearly demonstrated its life history under the various conditions obtaining.

In the saprophytic, free-growing state it forms on culture media a firm surface layer with abundant soft, white, loosely interwoven superficial growth. Under the conditions which have obtained, it is nonfructifying, is septate in a minority of filaments, and the aërial hyphæ separate into short cylindrical cells which may later become oval, suggesting the yeastlike fungi. It grows luxuriantly on most laboratory media, and all cultures develop a strong "yeasty" odor.

Under conditions of animal-tissue parasitism the fungus is extremely modified, appearing primarily as solid, usually round bodies of nuclear material, most of which are free in tissue spaces. These parasites are often identified with difficulty, and in many preparations may be overlooked. They usually multiply by a simple fission to form two bodies, though in some cases, having attained maximum size, a parasite will undergo irregular multiple fission of the nuclear portion to produce botryoid groups of small bodies of different sizes which are ultimately liberated by dissolution of the protoplasm.

In different conditions of artificial cultivation the basic tissue forms may undergo various changes. In cultures containing free blood or tissue elements, that is, in what may be considered semiparasitic environment, they continue to multiply as parasites, and on no ordinary medium is the saprophytic habit of growth recovered. In these cultures, however, often after developing a protoplasm, they undergo modification to the extent that they differentiate within themselves numbers of a peculiar, very small, hyaline spore-granule, this process being inaugurated in the nuclear portion which later loses its identity. Under favorable conditions some of these spores enlarge, become deeply staining, and repeat the cycle.

This type of endosporulation is, so far as I have been able to learn, without precedent. The spore-granules are different from bacterial spores in size, regularity, and the capability of withstanding unfavorable conditions except, perhaps, drying. Furthermore, but a small proportion of them has proved viable even under the most favorable circumstances. They are still more unlike any of the many types of spores familiar to mycologists. Hyaline granules seen in fungi—probably of the nature of ordinary lipoid and other protoplasmic granules (as in figs. 44 to 46)—have often been called endospores, though their germination has not been observed. In the described fungus the spore-granules are produced in an entirely different manner and have developed only under conditions of semiparasitism.

The large gelatinous bodies which develop in some cultures also seem to represent a condition of semiparasitism and are intermediate between the tissue forms and the free-growing fungus, since from them either of these may develop. The more or less gelatinous content of these large, sometimes almost cystlike bodies may produce: *a*, spore-granules alone; *b*, spore-granules and basic forms, larger and smaller; or *c*, basic forms alone. Under the most favorable conditions (in my experience only on properly made banana media), the basic forms thus produced within these bodies enlarge, become irregular in shape, and after two weeks develop the saprophytic mycelial fungus. This gelatinous body seems also to be very unusual, as I have found no mention of similar bodies in descriptions of other fungi.

A striking feature of the study of this fungus has been the difficulty encountered in converting the parasitic to the saprophytic form and the extreme contrast which these forms present. The difficulty of cultivation was not due to the lack of viability of the parasitic forms, since they multiplied fairly readily in certain artificial environment, but was simply due to the inability of the parasitic forms to readapt themselves to saprophytism. The real nature of the fungus and the significance of its tissue forms could not have been established had not the utilization of banana media accomplished its cultivation in free-growing form.

Little can be said of the botanical classification of the organism, pending further investigation of its relation to certain other fungi, particularly in the manner of its endosporulation. It seems likely that it will prove closely related to *Monilia*, though it is distinct from that genus.

The multiplicity of forms that this fungus may assume is unusual for a pathogenic fungus, though the forms themselves probably may be produced by other infectious fungi. Bodies similar to those here called the basic parasitic forms have been not infrequently described in other mycotic lesions, though in some instances they are described as "spores," while in most instances they have been thought to be products of degeneration of tissue and fungus cells. They appear, on the contrary, to be the basic multiplication form which a number of different fungi—how many cannot be stated—assume in adapting themselves to the parasitic rôle.

Fungi, in adapting themselves to conditions of animal tissue-cell metabolism, undergo extreme modification in structure and form. That these vegetable cells, so modified physically and chemically as to be able to abstract nutriment from the more dense body fluids and to utilize such material, may assume characteristics that make it almost impossible clearly to distinguish them by morphology or by staining reactions from the simpler types of body cells does not seem remarkable. It is, however, a fact that has not been generally recognized.

The clinical features of the described infection cannot be broadly discussed on the basis of a single case. An interesting feature that may be noted, however, is the wide distribution of a great number of small lesions, indicating a hematogenous dissemination. This is emphasized by the histology of the active lesion, in which are large vascular spaces many of which contain parasitic bodies. While tissue sections containing simple parasitic forms might be easily passed over without remark, the parasite-filled phagocytic cells and the botryoid clusters of various-sized bodies, which in my sections have been found only in the more active areas, are striking.

While no case clinically similar has been found in the literature, certain descriptions of the condition known as "botryomycosis hominis" and a few of those called, perhaps erroneously, "tropical inguinal granuloma" indicate some similarity of histologic findings with those of the present case.

In the small granulomatous skin tumors, first described by Poncet and Dor,(8) there are often to be seen small groups of bodies apparently like those here described. Whether rightfully or not, this condition has in recent years been classed by many authors as a staphylococcal granuloma. Very different, however, are the extensive lesions described as botryomycotic by Archibald(1) from the Sudan and by Castellani(3) from Ceylon. These are sometimes tumorlike and may even necessitate amputation of entire parts of the body. The former author found in sections groups of large and small bodies, free and in endothelial leucocytes, and also described and depicted a protoplasmic rim about some of the free bodies.

The typical "tropical inguinal granuloma" as described by Donovan,(5) Carter,(2) Conyers and Daniels,(4) Wise,(11) Flu,(6) and others is evidently a distinct infection, thought by many to be protozoic. However, certain cases that have been given this diagnosis may be of different nature. Such, for instance, are the three cases in American negroes reported by Grindon,(7) in sections from which the peculiar bodies of Donovan and of Carter were not found. In an illustration accompanying his article (his figure 6) is shown a photomicrograph of a section in which are discernible, both within the epidermis and in the connective tissue, many small, compact bodies and "cells" which, as well as can be made out, seem indistinguishable from any of the parasite bodies of the similar section in fig. 9 herewith. Strangman(10) claims to have isolated a complex fungus from cases of granuloma pudendi.

Obviously one cannot more than suggest the possibility that, so far as the microscopic appearances of the described lesions are concerned, some of these conditions are in some ways similar to the infection in this case.

CONCLUSIONS

From a case of a peculiar chronic skin infection there has been isolated an apparently hitherto undescribed fungus. Some of its parasitic forms are very difficult or impossible to identify in the lesions, as they have the ordinary staining reactions of nuclear material. In cultures, forms frequently develop that are easily overlooked as not significant. It was obtained free-growing with considerable difficulty, having regained its mycelial habit only on banana media, after which it grew luxuriantly.

Phases of its morphology having reference to the described basic form, entire-cell phase, gelatinous and vesicular-body formation, and reproduction by endosporulation are features not generally recognized as occurring among fungi. It is believed, however, that certain familiar fungi, apparently distinct but possibly related, may under similar conditions exhibit certain of these characteristics.

The histopathological features of the described infection suggest its relation to the so-called botryomycosis hominis and possibly to certain cases, described as granuloma inguinale.

The classification and the name of this new fungus are left in abeyance, to await the results of other studies now under way.

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ILLUSTRATIONS

PLATE I

FIG. 1. Numerous small lesions on back, with one frambœsial nodule.

2. Showing the deep infiltration of the lobe of the ear and the verrucous lesion of the margin.

3. Closer view (slightly larger than natural) of nodule in fig. 1.

4. External surface of right arm, showing numerous active lesions and edge of an extensive scar on the posterior surface.

5. Extensive keloidal cicatrix on left elbow. An active lesion later developed at the point indicated.

PLATE II

FIG. 6. Low magnification of granulomatous tissue from a typical active nodule, after Mallory's connective-tissue stain. The more active lesions under the epidermis photograph black because of great numbers of fuchsin-stained erythrocytes. × 25.

7. A moderately active focus, showing accumulation of various cells and the prominence of vascular spaces. Delafield's hæmatoxylin and eosin. × 125.

8. A group of basic forms apparently recently liberated. In the lower left corner is a half-grown parasite with loose protoplasm. × 1000.

9. Shows an indefinitely outlined phagocyte above and one free in a blood space below. Numerous free parasites are also present. In the lower right corner is a nude body (basic form). The arrow indicates a probable form which resembles a plasma cell. × 1000.

10. A phagocyte containing normal and degenerated parasites. Above is a parasite which has become irregularly outlined, apparently in preparation for subdivision. × 1000.

11. From smear of tissue scrapings. Two endothelial phagocytes containing numerous typical basic parasitic forms. One larger, free parasite also present. Giemsa's stain. × 1000.

12. A similar phagocyte with five basic forms. From the same preparation as fig. 11.

PLATE III

FIG. 13. An active focus illustrating the invasion of the epidermis by the infection. Many of the cells that resemble leucocytes are possibly parasites. The condition of the erythrocytes indicates direct connection with the blood channel in the chorium. A granule is budding off of the parasite (?) indicated. Ehrlich's iron hæmatoxylin stain. × 450.

14. Showing several parasites in various stages. A number of round forms, with and without protoplasm, are scattered throughout the preparation. At *a* a large parasite has segmented to produce basic parasitic forms, and at *b* are a number of these forms recently liberated. × 450.

PLATE IV

FIG. 15. From a Gram-stained smear from a primary culture on ordinary medium. Numerous fine, Gram-positive granules are division forms of the parasite. × 1000.

16. Similar to above, from another culture. × 700.

17. One appearance of an old, thin, gelatinous form, after Gram's stain. × 400.

18. A typical entire-cell form from culture in smeared blood. Giemsa's stain. × 1000.

19. Above, an entire-cell form with one refractile spore-granule in nucleus. Below, a later stage, nucleus indistinguishable, the whole containing many such granules. Field slightly out of focus. Giemsa's stain. × 1000.

20. Large, soft cell about to liberate group of spores. × 1000.

21. Unusually compact mass of spores. On account of plane of focus all but one appear black. Giemsa's stain. × 1000.

22. Débris in smear from culture, containing myriads of minute, free spore-granules, stained light blue in the preparation. A number of the larger are clearly discernible. In the center is a darkly stained oval body which corresponds to the basic form. Loeffler's methylene blue. × 825.

23. Group of spores beginning to develop. From culture in smeared blood. Giemsa's stain. × 1000.

24. Intermediate form containing large metachromatic granules. Developed from spores in smeared blood. Loeffler's methylene blue. × 1000.

25. Older protozoönlike cell. Giemsa's stain. × 1000.

26. Early stage of gelatinous body, developing from basic form. Giemsa's stain. × 750.

27. Intermediate stage of gelatinous body about to differentiate basic forms within itself. Giemsa's stain. × 400.

28. Group of basic forms produced within a gelatinous body. × 400.

PLATE V

FIG. 29. Mass of gelatinous bodies, the protoplasm of which has condensed to form numerous basic forms of various sizes. Somewhat earlier stage than in fig. 27. × 200.

30. Large body, from ten-day culture on banana cylinder, containing ordinary round basic forms and bizarre forms of similar material, apparently representing an attempt at mycelial growth. × 500.

31. A group of collapsed walls of banana-pulp cells, in smear of material planted on banana cylinder. These might be mistaken for vesicular forms of the gelatinous bodies. × 100.

FIGS. 32 and 33. Empty banana-pulp cells. × 400.

FIG. 34. Segments of aërial hyphæ, from free-growing culture on agar. Tendency to deeper staining at ends. Refractile granules in one segment. × 750.

35. Same as above, several cells showing metachromatic granules (black). × 750.

PLATE VI

FIG. 36. Five-day growth on banana cylinder, illustrating soft, tangled character of aërial growth.

FIGS. 37 and 38. Comparing two-day growth on nutrient banana agar and on banana cylinder.

FIG. 39. Firm "thimble" of growth, seven days on nutrient banana bouillon. Drawn up in tube and turned to demonstrate firmness.

40. Growth on plain bouillon, one week. Cottony aërial growth collapsed except over two small areas.

41. Development of mycelial segments on agar, without oxygen. Early vacuolation. × 450.

42. Similar to above. Several very refractile hyalinized segments. Extreme vacuolation of mother cells, which show growth from side. × 450.

43. Old mycelium from depths of bouillon. Highly refractile granules formed within the sheath. A delicate septum, to be seen only in occasional senile hyphæ, is indicated. × 450.

FIGS. 44 and 45. Two terminal bodies (chlamydospores) found in hanging-drop preparations. That in fig. 45 is not attached to the adjacent filament. × 450.

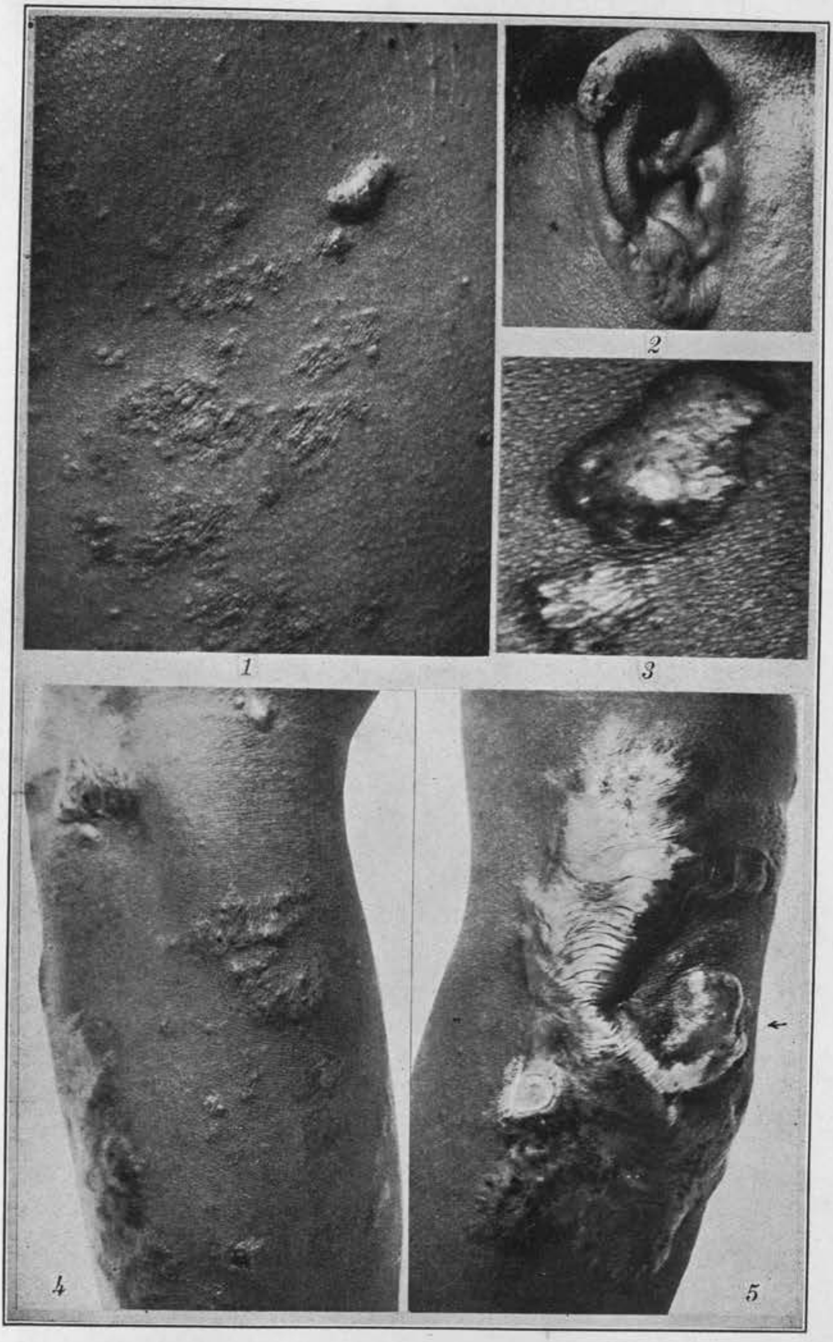
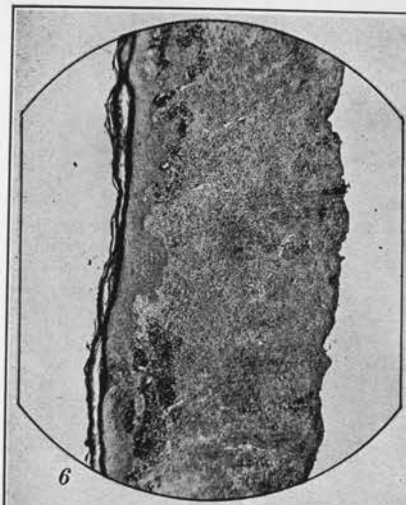


PLATE I.



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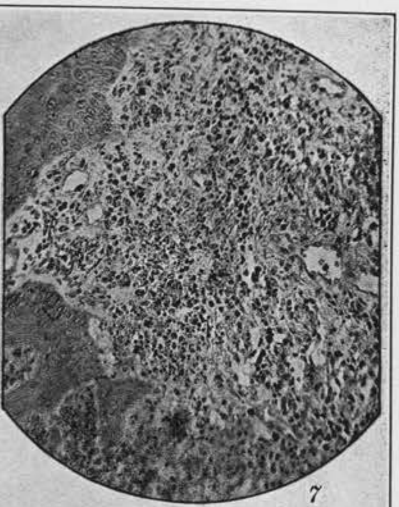

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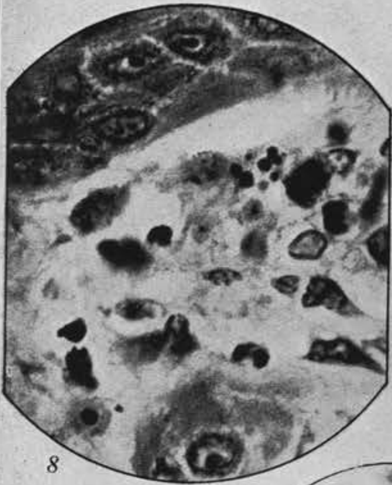

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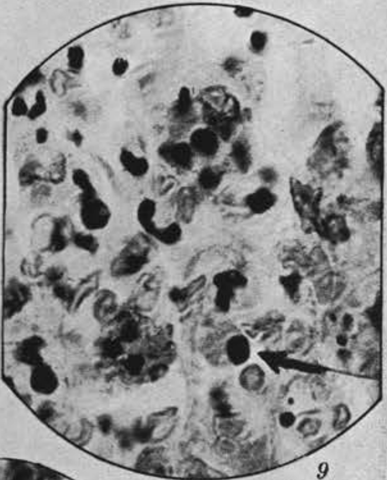

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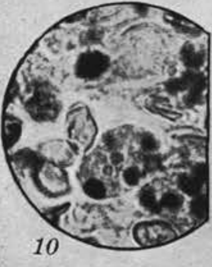

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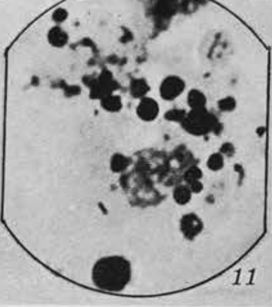

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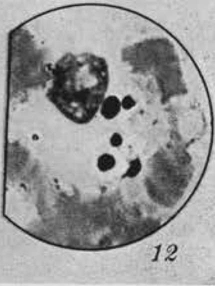

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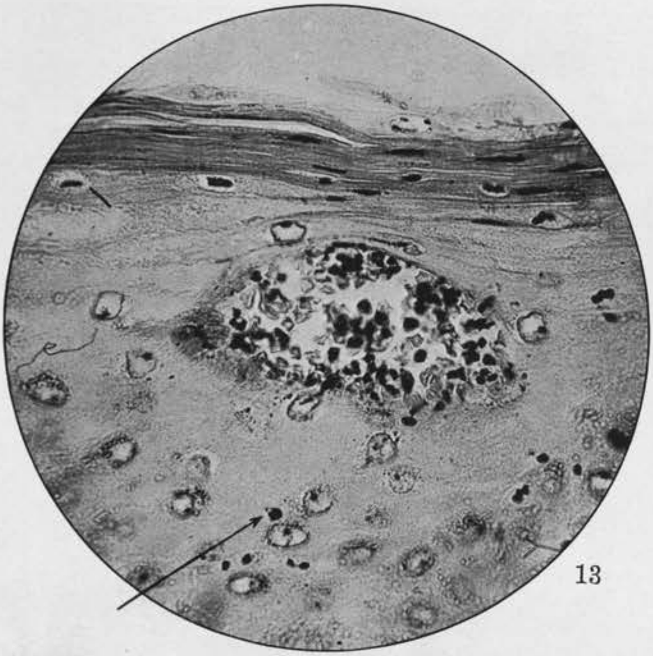
PLATE II.



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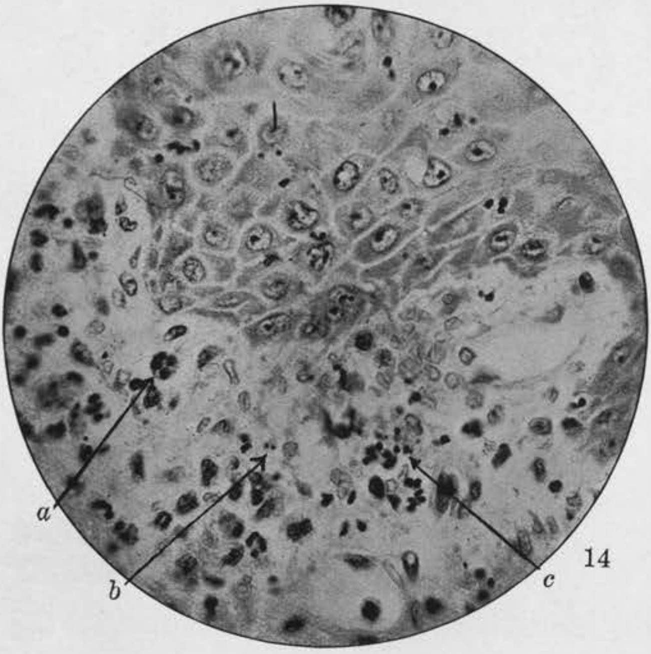

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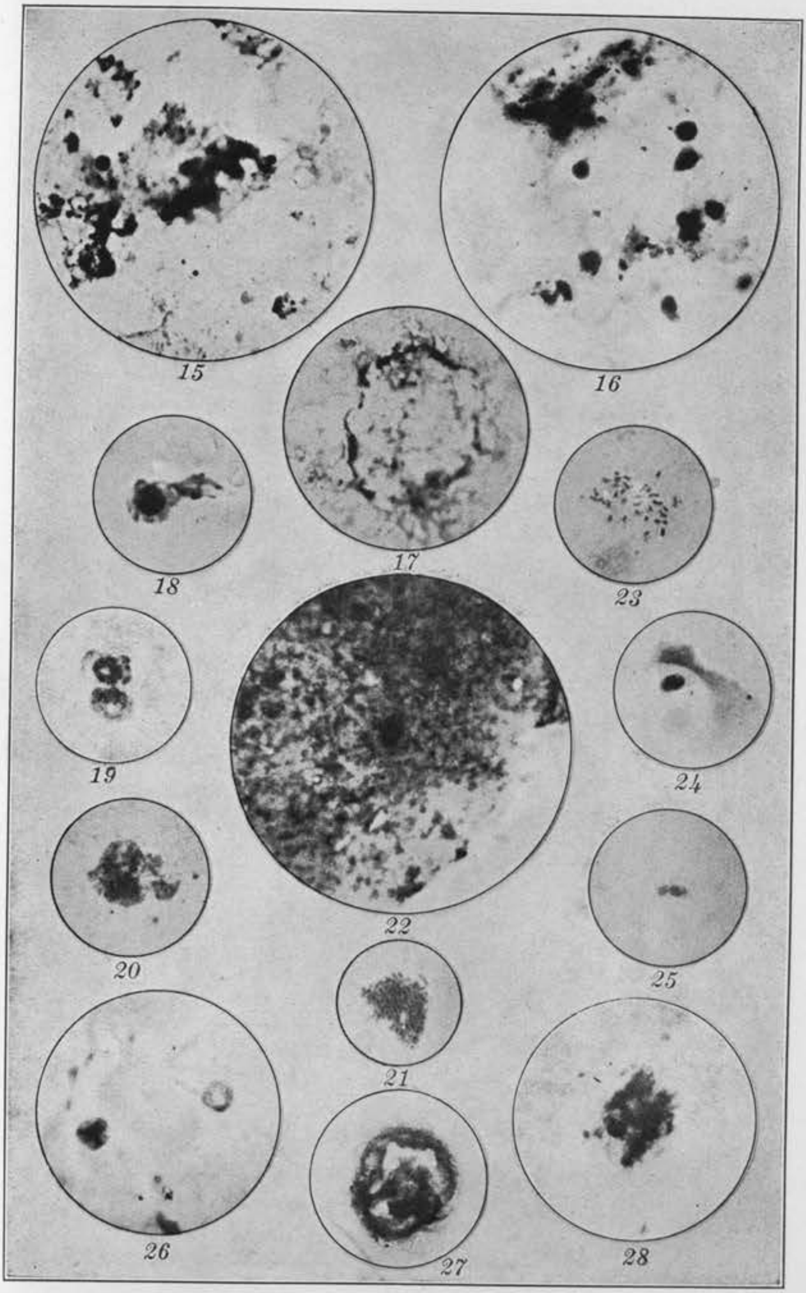
PLATE III.



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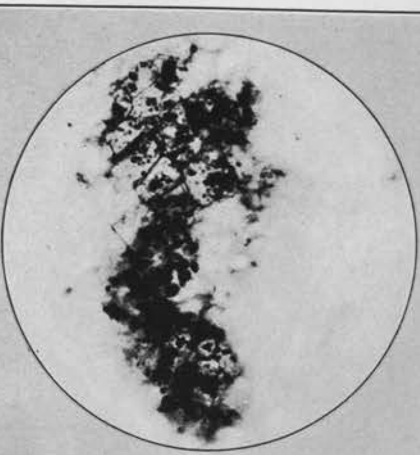

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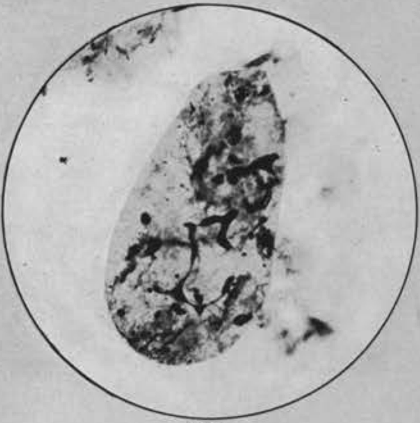

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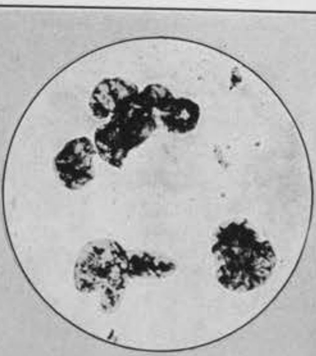

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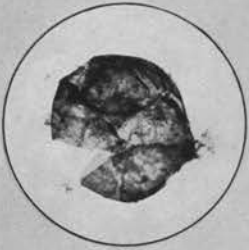

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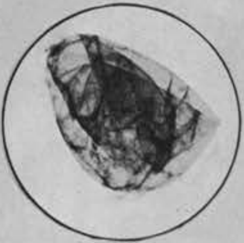

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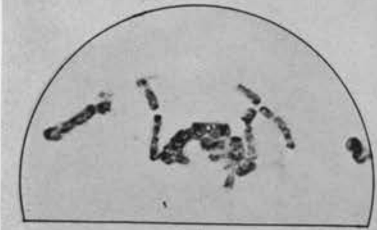

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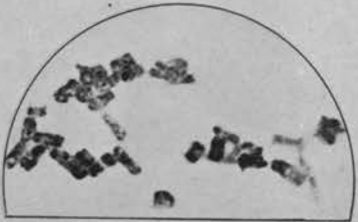

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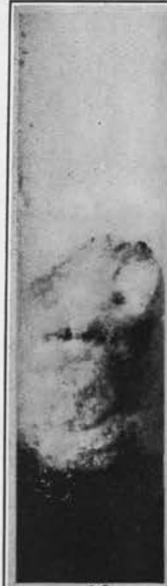
PLATE V.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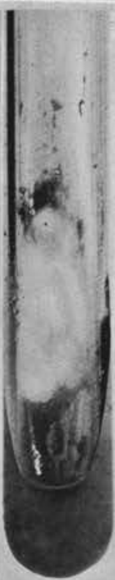
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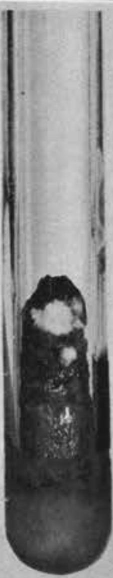
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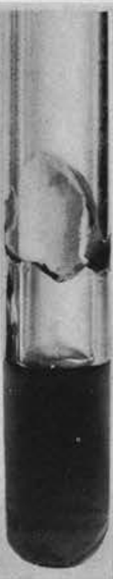
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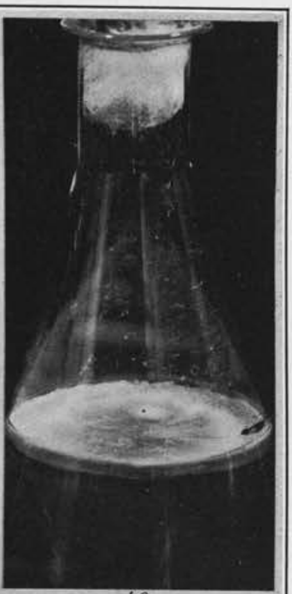
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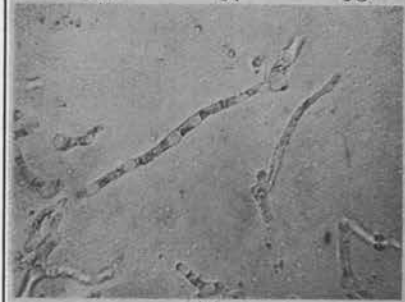
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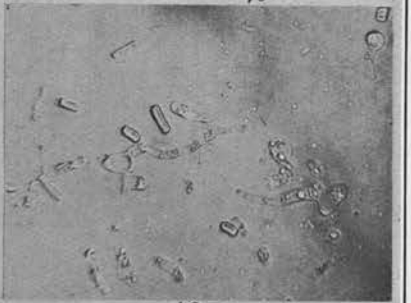
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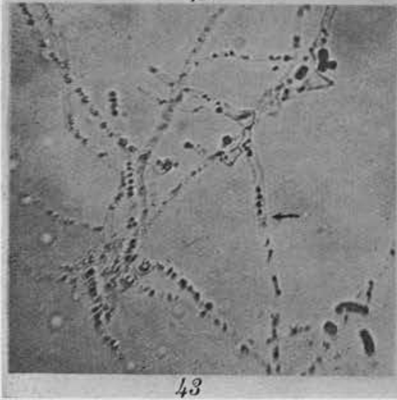
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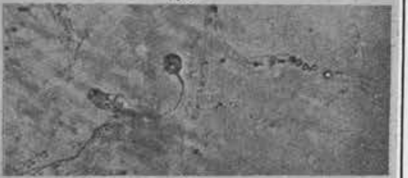
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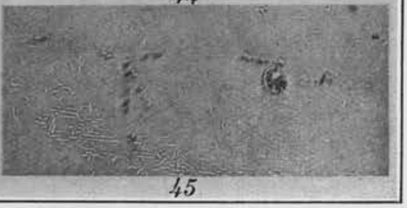
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PLATE VI.

A FATAL PARASITIC INFESTATION IN A HERD OF CATTLE AND GOATS IN AMBOS CAMARINES PROVINCE[1]

By WILLIAM HUTCHINS BOYNTON and LAWRENCE D. WHARTON

(*From the Bureau of Agriculture and the University of the Philippines*)

In January, 1916, W. H. Boynton was sent to Ambos Camarines Province to investigate the cause of the death of a large number of animals on a plantation near the town of Minalabag. These animals were kept for breeding purposes in a large pasture, a part of which was high land and a part low rice-paddy land.

The herd was composed of 90 animals immunized by the simultaneous method against rinderpest on March 1, 1915, and of 55 animals that had not been immunized against rinderpest.

The animals that were not immunized were brought from Masbate Island and arrived at the plantation on September 20, 1915. The immunized and the nonimmune animals were kept together.

The owner of the cattle maintained a strict quarantine against outside animals, as there was rinderpest in some of the neighboring districts and he was afraid the nonimmune animals would contract the disease. Every evening the cattle were placed under cover, and smudges were burned around this inclosure during the night to keep off mosquitoes, which are very plentiful in that locality. The animals received an abundance of green feed from the low pasture land. On account of the precautions taken against rinderpest infection, both in having a large number of immunized animals and in quarantining against outside animals, and also on account of the care which the animals received, it was expected that they would thrive.

Practically all the animals were in good condition when they arrived at the plantation. After a time many of them began to lose weight, even with the feed and care which they received, and as the caretaker states, they became thinner and thinner and finally died, although they ate well until just before death.

A considerable number died during and just after the heavy storms, which indicated that they did not have vitality enough to withstand these storms.

There were also 60 head of goats on the place, and at the time of Boynton's visit all but 11 had died and one of

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[1] Reprinted from *Phil. Agr. Rev.* (1916), 9, 348.

these was sick. The sick goat was emaciated, was very anæmic in appearance, stood in the sun with his back arched and head down, had a slight diarrhœa, and possessed very little life, moving with difficulty when urged. The caretaker stated that this was the general appearance of the greater percentage of those that had died.

This animal was killed and autopsied. The blood was very anæmic in appearance. There was practically no fat in the peritoneal cavity, the organs were pale, the mesenteric lymphatics were somewhat enlarged and soft, and upon section a watery serous fluid oozed from them. The fourth stomach and the duodenum contained myriads of worms—red from imbibing the animal's blood—and small petechiæ were scattered over the mucous membrane of these organs. There were many worms of a different character in the cæcum and in the colon. The enormous number of these intestinal parasites was undoubtedly accountable for the condition of this animal. Specimens of these worms were preserved and later were identified by L. D. Wharton.

There had been no deaths among the cattle for some time prior to Boynton's visit, but two animals were very thin, and the caretaker stated that they had exactly the same appearance as those that had died. For this reason one was killed and autopsied to see if the cause of the condition could be ascertained.

Upon autopsy the internal organs were found to be anæmic; the heart, the kidneys, the spleen, and the liver were normal, except for their pale appearance. Worms were found in the bronchioles of the lungs, and there was a slight pneumonia in the left principal lobe. Many worms were found in the fourth stomach, the small intestines, the cæcum, and the upper part of the colon. There was a marked catarrhal enteritis in the ileum and numerous petechiæ. In many instances the worms were dislodged from the mucosa with some difficulty. Specimens of the worms found in the various parts were preserved and later were identified by Wharton.

The large number of these intestinal parasites was undoubtedly the direct cause of the emaciated and anæmic condition of this animal.

From the autopsies of the two animals picked out by the caretaker as being identical in appearance with many of the animals that had died, it is probable that a majority of the latter were also heavily infested with intestinal parasites and that these parasites were the direct cause of death.

It will be noted from Table I that 11 cattle died during the first storm on October 23 and that 14 died during the second storm on November 3. This indicates that their vitality was so low that the animals could not withstand exposure when infested to such an extent with these intestinal parasites.

The caretaker was directed to transfer the entire herd to grazing lands on high ground, and this was done. On April 15, 1916, the Bureau of Agriculture was notified that only two of the cattle had died after being transferred to the high ground, and that the whole herd was in good condition with the exception of one animal which had not completely recovered. This fact demonstrated that the animals had become infested on the low-lying grazing grounds.

TABLE I.—*Showing the number of deaths from intestinal parasites in a herd of cattle and goats in Ambos Camarines Province. Total deaths, 69.*

Date.	Died.		Date.	Died.	
	Immunized.	Nonimmunized.[a]		Immunized.	Nonimmunized.[a]
1915.			1915.		
Mar. 6	1		Oct. 15		1
Mar. 28	1		Oct. 20		1
Apr. 17	1		Oct. 22		1
May 9	1		Oct. 23[b]	5	6
June 2	3		Nov. 3[c]	10	4
June 3	1		Dec. 3	1	
June 9	1		Dec. 4		1
Aug. 1-31	9		Dec. 7	2	1
Sept. 20		1	Dec. 8	1	
Sept. 25	2	2	Dec. 9		1
Sept. 28	2		Dec. 22		1
Oct. 7		2	Dec. 23[d]	2	
Oct. 12		1			
Oct. 13		3	Total	43	26

[a] The nonimmunized animals arrived from Masbate on September 20, 1915.
[b] First typhoon.
[c] Second typhoon.
[d] Third typhoon.

The collection of worms made by Boynton was found to contain 8 species, 7 from the cow and 2 from the goat. One species, *Haemonchus contortus,* was found in both individuals. The species, as far as they can be identified with the limited literature at hand, are as follows:

NEMATODES

STRONGYLIDÆ

Bunostomum phlebotomum (Railliet, 1902).

Strongylus radiatus, Dochmius radiatus, Uncinaria radiata, Monodontus phlebotomus.

These are small white worms with a large, armed buccal capsule, bent dorsally. There is a short, dorsal buccal tooth; a pair of ventral buccal teeth; and a pair of small, subventral lancets; all attached to the base of the capsule. There are six circumoral papillæ and a pair of short, blunt cervical papillæ opposite the anterior portion of the œsophagus. The œsophagus is enlarged at the posterior end.

The males are 10 to 14 millimeters long and about 0.5 millimeter in thickness. The dorsal lobe of the bursa copulatrix is shorter than the lateral lobes. The dorsal ray is asymmetrical, coming off from the base of the left lateral ray and giving off the externolaterals asymmetrically. The spicules are long and filiform.

The females are 15 to 20 millimeters in length and a little thicker than the males. The posterior end of the body is attenuated and terminates in a slender, bluntly pointed tail behind the anus. The vulva is situated a little in front of the middle of the body.

The above were collected from the ileum and the fourth stomach of a cow (*Bos taurus*) at Minalabag, Camarines, and are listed as specimen No. 110, helminthological collection, College of Medicine and Surgery.

Oesophagostomum columbianum (Curtice, 1890).

This is the common *Oesophagostomum* of sheep and goats. It undergoes its development in nodules in the walls of the intestines and leaves these nodules only when it has reached the adult stage, at which time it passes into the lumen of the intestine, where copulation and egg-laying occur.

The mouth is surrounded by a slightly inflated mouth collar. There are well-developed lateral membranes extending posteriorly from the transverse groove along the whole length of the body. A pair of cervical papillæ are present in front of the middle of the œsophagus.

The males are 10 to 16 millimeters long and have a bursa. The females are 14 to 20 millimeters long with the vulva close to the end of the tail.

The above were collected from the intestine of a goat at Minalabag, Camarines, and are listed as specimen No. 106, helminthological collection, College of Medicine and Surgery.

Oesophagostomum sp.

Worms of this genus were also collected from the cow. While they more closely resemble *O. radiatum* than any other described form, it is believed that they should be placed in a separate species. The mouth collar is more prominent than in *O. radiatum*, the cervical inflation of the cuticle is strongly pronounced, and the body is slightly larger than that of *O. radiatum*. The externodorsal rays of the bursa of the males originate very close to the root of the dorsal ray; and the external divisions of the dorsal ray are knob-shaped, while the internal divisions are very long and slender.

The above were collected from the cæcum of a cow, at Minalabag, Camarines, and are listed as specimen No. 104, helminthological collection, College of Medicine and Surgery.

TRICHOSTRONGYLIDÆ

Haemonchus contortus (Rudolphi, 1803) Cobb, 1898.

Strongylus contortus.

This is the common "wire worm" of cattle, sheep, and goats. The head is small and bears a minute buccal lancet. There are two cervical spines about 0.3 to 0.4 millimeter from the anterior end of the body. The œsophagus is claviform. The body is long and slender and is attenuated toward both ends.

These worms were found in the fourth stomach of both the goat and the cow at Minalabag, Camarines, and are listed as specimens Nos. 102 and 108, helminthological collection, College of Medicine and Surgery.

Nematode sp.—Several female worms, 30 to 35 millimeters in length and having a diameter of about 0.6 millimeter, were collected from the fourth stomach of the cow. They probably belong to the family Trichostrongylidæ, although they could not be identified further. They are filled with large thin-shelled eggs averaging 107 microns in length by 44 microns in diameter. The vulva is situated about 1 millimeter in front of the anus, and the tail is short and is sharply pointed behind the anus. They are listed as specimen No. 115, helminthological collection, College of Medicine and Surgery.

TRICHINELLIDÆ

Trichuris ovis (Abildgaard, 1795) Smith, 1908.

Trichocephalus affinis.

This is the common whipworm of cattle and sheep. Specimens measure from 45 to 70 millimeters in length, the anterior slender portion of the body making up from two thirds to three fourths of the whole length.

The posterior end of the male is curved spirally and bears a single spicule surrounded by a spiny sheath with a bulbous enlargement at the end. The posterior end of the female is straight and is filled with lemon-shaped eggs.

These were collected from the cæcum of a cow at Minalabag, Camarines, and are listed as specimen No. 103, helminthological collection, College of Medicine and Surgery.

FILARIIDÆ

Filaria labiato-papillosa.

These are long, slender, threadlike white worms with a terminal mouth surrounded by a chitinous ring bearing two papillalike processes.

The males are 4 centimeters long with a spiral, pointed tail, bearing four pairs of preanal and five postanal papillæ and two unequal spicules.

The females are 6 to 7 centimeters long. The posterior end is slightly curved and terminates in a fasciculus of small blunt points preceded by two thick, cortical, lateral papillæ.

These worms were found free in the abdominal cavity and in the lungs of a cow, Minalabag, Camarines, and are listed as specimen No. 114, helminthological collection, College of Medicine and Surgery.

TREMATODES

Paramphistomum sp.

A single small specimen of this genus was found in the stomach of the cow.

It is immature, and so the species could not be determined. It is listed as specimen No. 112, helminthological collection, College of Medicine and Surgery.

REVIEWS

The | Non-surgical | Treatment | of | Intestinal Stasis | and Constipation | compiled by | Robert H. Ferguson, M. D., Sc. D. | also an important announcement | regarding liquid petrolatum | published for the physician and surgeon by | E. R. Squibb & Sons, New York | Medical Department | MCMXVI | Cloth, pp. 1–109.

The title of this little volume is misleading. A few pages are devoted to the definition of intestinal stasis, the cause of it, its importance, and its remote effects. Most of the volume is a collection of favorable statements concerning liquid petrolatum as a remedy for intestinal stasis. The statements are excerpts from published articles which may or may not include records of experiments, but with the exception of the mention of four cases, the book itself offers no experimental data.

J. L. BOOTH.

Diseases | of the | Digestive Tract | and | Their Treatment | by | A. Everett Austin, A. M., M. D. | [11 lines] | St. Louis | C. V. Mosby Company | 1916 | Cloth, pp. 1–552. Price $5.50.

A striking feature of this pretentious volume is the very general lack of proportion. Nearly one third of it is taken up with the various methods of examination and diagnosis, with special emphasis on the X-ray. The physiology of digestion and the chemistry of the digestive tract, both in health and disease, are elaborately detailed. On the other hand, barring a few words on the examination of the mouth, teeth, and pharynx, the part of the tract above the stomach is completely ignored, while the diseases of the tonsils and pyorrhœa alveolaris are not mentioned.

As a contrast, seven pages and six illustrations are given to the stomach tube and its administration, while one third of a page suffices for *Entamœba coli* and *E. histolytica* and the havoc wrought by them. No other protozoa are mentioned. The Shiga-Flexner group of dysentery bacilli are dismissed with a three-line notice. Typhoid enteritis is not discussed.

Intestinal parasites, the diseases caused by them, and their treatment occupy only six pages, of which one is considered sufficient for hookworm infection.

The illustrations of *Oxyuris vermicularis* and *Ankylostoma duodenale,* marked natural size, are two and one-half times too large and do not remotely resemble these worms.

J. L. BOOTH.

Local and Regional | Anesthesia | [4 lines] | by | Carroll W. Allen, M. D. | [3 lines] | with an introduction by | Rudolph Matas, M. D. | [2 lines] | illustrated | Philadelphia and London | W. B. Saunders Company | 1915 | Pages 1–625. Cloth, $6 net; half morocco, $7.50 net.

Allen's Local and Regional Anesthesia contains a most extensive work on local anesthesia. This branch of modern surgery has assumed a foremost importance of late years, and Dr. Allen's work is a most valuable contribution to the science. The description and technic is easy and comprehensive and well repays study.

A. VAZQUEZ.

Surgery | of the | Blood Vessels | by | J. Shelton Horsley, M. D., F. A. C. S. | [5 lines] | illustrated | St. Louis | C. V. Mosby Company | 1915 | Cloth, pp. 1–304. Price $4.

Horley's Surgery of the Blood Vessels contains some of the latest work on this subject and opens a fresh field for further extensive research. The style is fluent, comprehensive, and interesting. The book will be most useful to the modern surgeon.

A. VAZQUEZ.

INDEX

A

ALBERT, JOSÉ, review of Royster's A Handbook of Infant Feeding, 185.
Allen, Carroll W., *see* Reviews (book).
Allolobophora trapezoides, 110.
Ambos Camarines, a fatal parasitic infestation in a herd of cattle and goats in, 285.
Amphioxus, 110.
Anamirta cocculus, 203.
Ankylostoma trigonocephalum, 25.
Apomorphine, the use of, to remove foreign bodies from the respiratory passages, 51.
Ascaris, 27.
Austin, A. Everett, *see* Reviews (book).

B

Bacillus coli, 63.
diphtheriæ, 63, 168.
dysenteriæ, 63.
proteus, 168.
pyocyaneus, 67.
tetani, 63.
tuberculosis, 63.
typhosus, 63.
Bacillus pestis, carbohydrate fermentation by, comparing certain American and oriental strains, 159.
Bacteriological examinations of swimming pools in Manila, 63.
Bile, the influence of, upon the duration of the state of cholera carriers in experimental animals, 157.
Blood-pressure picture of the Filipinos, 135.
BOOTH, J. L., review of Austin's Diseases of the Digestive Tract and Their Treatment, 291; review of Ferguson's The Non-surgical Treatment of Intestinal Stasis and Constipation, 291.
Bos taurus, 288.
Bothriocephalus latus, 27.
BOYNTON, WILLIAM HUTCHINS, Rinderpest in swine with experiments upon its transmission from cattle and carabaos to swine and vice versa, 215.
BOYNTON, WILLIAM HUTCHINS, and WHARTON, LAURENCE D., A fatal parasitic infestation in a herd of cattle and goats in Ambos Camarines Province, 285.
BRILL, H. C., review of Fantus's Candy Medication, 187.
Brooke, Gilbert E., *see* Reviews (book).
Brooks, Herbert Thomas, *see* Reviews (book).
BULATAO, EMILIO, *see* CONCEPCIÓN, ISABELO, 135.
Bunostomum phlebotomum, 288.

C

Cabot, Richard C., *see* Reviews (book).
Canis familiaris, 24.
Carabaos and cattle, rinderpest in swine with experiments upon its transmission from, to swine and vice versa, 215.
Carbohydrate fermentation by Bacillus pestis, comparing certain American and oriental strains, 159.
Cattle and carabaos, rinderpest in swine with experiments upon its transmission from, to swine and vice versa, 215.
Cattle and goats, a fatal parasitic infestation in a herd of, 285.
Cholera carriers, the influence of bile upon the duration of the state of, in experimental animals, 157.
Cholera culture, the relation between the amount of, injected into the gall bladder and the state of cholera carriers in experimental animals, 153.
Colerinas, 205.
CONCEPCIÓN, ISABELO, *see* GIBSON, R. B., 119.
CONCEPCIÓN, ISABELO, and BULATAO, EMILIO, Blood-pressure picture of the Filipinos, 135.
Cows' milk, influence of fresh and autoclaved, on the development of neuritis in animals, 119.
Crime, relation of systolic pressure to, 145.
CROWELL, B. C., review of Wadsworth's Post-mortem Examinations, 187.
Cryptoceptes trichodestes, 25.
Ctenocephalis canis, 24.
felis, 24.

D

Datura alba, 203.
Davis, Carl Henry, *see* Reviews (book).
DE LA PAZ, DANIEL, *see* GUERRERO, LUIS E., 203.
DE LA PAZ, DANIEL, and GARCÍA, FAUSTINO, An experimental study on the use of apomorphine to remove foreign bodies from the respiratory passages, 51.
Dioscorea daemona, 203.
Dipylidium caninum, a case of infestation with, 19.
Dochmius radiatus, 288.
Duck twins, on the development of twins and other polyembryos with special reference to to four sets of, 109.

F

Fantus, Bernard, *see* Reviews (book).
Felis catis, 24.
Ferguson, Robert H., *see* Reviews (book).
Fermentation, carbohydrate, by Bacillus pestis, comparing certain American and oriental strains, 159.
Filaria labiato-papillosa, 290.

Filariidæ, 290.
Filipinos, blood-pressure picture of the, 135.
Fungus, cultivation of a pathogenic, which exhibits botryoid and leucocytelike parasitic forms, 267.

G

GABEL, CHARLES E., Bacteriological examinations of swimming pools in Manila, 63; review of Schneider's Bacteriological Methods in Food and Drugs Laboratories, 107.
Gall bladder, the relation between the amount of cholera culture injected into the, and the state of cholera carriers in experimental animals, 153.
GARCÍA, ARTURO, Congenital bilateral absence of kidneys in a 140-millimeter pig embryo, 191.
GARCÍA FAUSTINO, *see* DE LA PAZ, DANIEL, 51.
GIBSON, R. B., review of Ott's Fever its Thermotaxis and Metabolism, 107.
GIBSON, R. B., and CONCEPCIÓN, ISABELO, The influence of fresh and autoclaved cows' milk on the development of neuritis in animals, 119.
Goats and cattle, a fatal parasitic infestation in a herd of, 285.
GUERRERO, ALFREDO L., *see* GUERRERO, LUIS E., 203.
GUERRERO, LUIS E., DE LA PAZ, D., and GUERRERO, ALFREDO, L., Poisoning by Illicium religiosum Siebold, 203.
GUTIERREZ, P., review of Cabot's Differential Diagnosis, 184.

H

Haemonchus contortus, 289.
HERNANDEZ, ADN., review of King's Farmers of Forty Centuries, 105.
Hiss's serum water, litmus agar, and bouillon, discrepancies of fermentations with, 159.
Hoffman, Frederick L., *see* Reviews (book).
Horsley, J. Shelton, *see* Reviews (book).
Hymenolepis nana, 27.

I

Infestation, a fatal parasitic, in a herd of cattle and goats in Ambos Camarines Province, 285.
Illicium anisatum, 205.
    floridanum, 210.
    griffithii, 210.
    majus, 210.
    montanum, 211.
    parviflorum, 210.
    philippinense, 211.
Illicium religiosum Siebold, poisoning by, 203.

J

Jatropha curcus, 203.
    multifida, 203.
JOHNSTON, JOHN A., review of Brooke's Aids to Tropical Medicine, 108.

K

Katbalonga, 203.
KEATING, T. F., review of Brooks's Diagnostic Methods, 186.
Kidneys, congenital bilateral absence of, in a 140-millimeter pig embryo, 191.
King, F. H., *see* Reviews (book).
Kolmer, John A., *see* Reviews (book).

M

Maná, 203.
MENDOZA-GUAZON, MARIA PAZ, A case of infestation with Dipylidium caninum, 19.
Micrococcus gonorrheæ, 63.
Milk, influence of fresh and autoclaved cows', on the development of neuritis in animals, 119.
Monilia, 278.
Monodontus phlebotomus, 288.
Morphine and opium habitués, blood pressure in, 146.

N

Namí, 203.
Nematodes, 288.
Neuritis, influence of fresh and autoclaved cows' milk on the development of, in animals, 119.
Niles, George M., *see* Reviews (book).

O

Oesophagostomum columbianum, 288.
    radiatum, 289.
    sp., 289.
Opium and morphine habitués, blood pressure in 146.
Ott, Isaac, *see* Reviews (book).

P

Paramphistomum sp., 290.
Parasitic infestation, fatal, in a herd of cattle and goats in Ambos Camarines Province, 285.
Pig embryo, congenital bilateral absence of kidneys in a 140-millimeter, 191.
Poisoning by Illicium religiosum Siebold, 203.
Polyembryos, on the development of twins and other, with special reference to four sets of duck twins, 109.
Pools, swimming, bacteriological examinations of, in Manila, 63.
Pulex irritans, 25.
    serraticeps, 25.

R

Respiratory passages, the use of apomorphine to remove foreign bodies from the, 51.
REVIEWS (BOOK):
    Allen, Carroll W., Local and Regional Anesthesia, 292.
    Austin, A. Everett, Diseases of the Digestive Tract and Their Treatment, 291.
    Brooke, Gilbert E., Aids to Tropical Medicine, 108.

REVIEWS (BOOK)—Continued.
  Brooks, Herbert Thomas, Diagnostic Methods, 186.
  Cabot, Richard C., Differential Diagnosis, 184.
  Davis, Carl Henry, Painless Childbirth, Eutocia and Nitrous Oxid-oxygen Analgesia, 184.
  Fantus, Bernard, Candy Medication, 187.
  Ferguson, Robert H., The Non-surgical Treatment of Intestinal Stasis and Constipation, 291.
  Hoffman, Frederick L., The Mortality from Cancer throughout the World, 183.
  Horsley, J. Shelton, Surgery of the Blood Vessels, 292.
  King, F. H., Farmers of Forty Centuries, 105.
  Kolmer, John A., A Practical Text-book of Infection, Immunity and Specific Therapy, 105.
  Niles, George M., Pellagra, 188.
  Ott, Isaac, Fever its Thermotaxis and Metabolism, 107.
  Royster, Lawrence T., A Handbook of Infant Feeding, 185.
  Schneider, Albert, Bacteriological Methods in Food and Drugs Laboratories, 107.
  Wadsworth, William S., Post-mortem Examinations, 187.
Rinderpest in swine with experiments upon its transmission from cattle and carabaos to swine and vice versa, 215.
Royster, Lawrence T., *see* Reviews (book).
RUEDIGER, E. R., Hæmolysis by human serum, 33; Preservation of human serum for Wassermann reaction, 1; review of Kolmer's A Practical Text-book of Infection, Immunity and Specific Therapy, 105; Wassermann reaction with glycerinated human serum, 87.
RUTH, EDWARD S., On the development of twins and other polyembryos with special reference to four sets of duck twins, 109.

S

Salabat, 205.
Salagó, 203.
Sanki, 203.
Schneider, Albert, *see* Reviews (book).
SCHÖBL, OTTO, The influence of bile upon the duration of the state of cholera carriers in experimental animals, 157; The relation between the amount of cholera culture injected into the gall bladder and the state of cholera carriers in experimental animals, 153.
Serum, hæmolysis by human, 33.
Serum, preservation of human, for Wassermann reaction, 1.
Serum, Wassermann reaction with glycerinated human, 87.
Spirillum choleræ, 68.
Streptococcus pyogenes, 269.
Strongylidæ, 288.
Strongylus radiatus, 288.
Strychnos ignatii, 203.
Suma, 203.
Swimming pools, bacteriological examinations of, in Manila, 63.
Swine, rinderpest in, with experiments upon its transmission from cattle and carabaos to swine and vice versa, 215.

T

Tænia, 26.
  asiatica, 27.
  cucumerina, 27.
  saginata, 27.
  solium, 27.
Tahu, 205.
Talampunay, 203.
Trematodes, 290.
Trichinellidæ, 289.
Trichocephalus affinis, 289.
Trichodectes canis, 24.
  rostratis, 24.
Trichostrongylidæ, 289.
Trichuris ovis, 289.
Tuba, 203.
Twins and other polyembryos, on the development of, with special reference to four sets of duck twins, 109.

U

Uncinaria radiata, 288.

V

VAZQUEZ, A., review of Allen's Local and Regional Anesthesia, 292; review of Horsley's Surgery of the Blood Vessels, 292.

W

WADE, H. WINDSOR, Carbohydrate fermentation by Bacillus pestis, comparing certain American and oriental strains, with analysis of discrepancies of fermentations with Hiss's serum water, litmus agar, and bouillon, 159; Cultivation of a pathogenic fungus which exhibits botryoid and leucocytelike parasitic forms, 267; review of Davis's Painless Childbirth, Eutocia and Nitrous Oxid-oxygen Analgesia, 184; review of Niles's Pellagra, 188.
Wadsworth, William S., *see* Reviews (book).
Wassermann reaction, preservation of human serum for, 1.
Wassermann reaction with glycerinated human serum, 87.
WHARTON, LAURENCE D., *see* BOYNTON, WILLIAM HUTCHINS, 285.
Wikstroemia ovata, 203.

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